PRODUCTION OF STERIGMATOCYSTIN BY SOME SPECIES AND VARIETIES OF *ASPERGILLUS NIDULANS* GROUP

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SUMMARY. — A total of 65 isolates of Aspergilli belonging to 4 species and 3 varieties of *A. nidulans* group were examined for production of sterigmatocystin when grown on Czapek's medium. All the isolates tested belonging to *A. nidulans*, *A. nidulans* var. *latus*, *A. quadrilineatus*, *A. rugulosus* and *A. violaceus* proved to be sterigmatocystin-producers. The mycological characters of the strains with high sterigmatocystin productivity were described. *A. nidulans* var. *latus*, *A. violaceus* have not been previously reported as sterigmatocystin-producers. The compound was never detected when the different isolates of *A. nidulans* var. *dentatus* and *A. nidulans* var. *acristatus* were cultivated on different media and at different temperatures and incubation periods.

I. — INTRODUCTION

The secondary metabolites of fungi known as mycotoxins have gained considerable importance during the past two decades as health hazards to animals and man (PURCHASE, 1974).

Sterigmatocystin, a metabolite of *Aspergillus versicolor* (Vuil.) Tiraboschi, consists of a xanthon nucleus attached to a bifuran structure and bears a close structural relationship to aflatoxin B_1 (SCHROEDER & KELTON, 1975). Sterigmatocystin is carcinogenic, but aflatoxin is 250 times more effective in inducing tumors (DICKENS et al., 1966). However, sterigmatocystin is produced in larger quantity than aflatoxin, up to 1.2 gm/kg of substrate (HOLZAPFEL et al., 1966) by fungi such *Aspergillus* sp., *Penicillium* sp., and *Bipolaris* sp. and therefore must be considered potentially hazardous to human and animals.

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The «*A. nidulans* group» is a large group including 19 species and 5 varieties (RAPER & FENNELL, 1965). The species of current mycotoxigenic concern are *A. nidulans* (Eidam) Wint. and *A. rugulosus* Thom et Raper. Both produce sterigmatocystin (BALLANTINE et al., 1965; HOLZAPFEL et al., 1966 and ISHIDA et al., 1972). Recently, production of sterigmatocystin by two species of *A. nidulans* group namely, *A. variecolor* Berk. et Br. and *A. unguis* (Emile-Neil et Gaudin) Thom et Raper was reported (CHEXAL et al., 1975 and MISLIVEC et al., 1975).

The present investigation was aimed at testing the production of sterigmatocystin by some members of the *A. nidulans* group. The relation between sterigmatocystin production and mycological characters of 65 isolates belonging to 4 species and 3 varieties of the *A. nidulans* group was also elucidated.

II. – MATERIALS AND METHODS

The test fungi were isolated in this laboratory over 2-years period from soil, seeds and cereal grain (ABDEL-HAFEZ & ABDEL-KADER, 1980; ABDEL-KADER et al., 1979; EL-HISSY et al., 1980; MOUBASHER & ABDEL-HAFEZ, 1978; MOUBASHER et al., 1979). Morphological study was made on Czapek's medium after incubation at 28°C for 12 days. For sterigmatocystin production, the fungi were cultivated on Czapek's liquid medium. YES medium (2% yeast extract + 15% sucrose) (DAVIS et al., 1966) and Czapek medium (glucose, 40.0; peptone, 1.0; yeast extract, 1.0; $MgSO_4$, 7 H_2O, 1.0 KH_2PO_4, 0.7 and asparagine, 0.7; g/l (CZAPEK et al., 1964), were also used. Fifty ml of medium were dispensed into each of several 250 -ml Erlenmeyer flasks and autoclaved (121°C, 20 min). After inoculation by particular fungi they were incubated at 28°C without shaking for 10 days. Each isolate was cultivated in triplicate. Detection of aflatoxin was made by the method previously reported by PONS & GOLDBLATT (1965). The identity and quantity of sterigmatocystin in the extracts were determined by thin-layer chromatography by the method of SCHROEDER & KELTON (1975).

III. – RESULTS

Sixty five isolates belonging to 4 species and 3 varieties of *A. nidulans* group were examined for the production of sterigmatocystin on Czapek's medium. *A. nidulans* was represented by 15 isolates. All produced sterigmatocystin, in the range 48 to 495 mg/l. The common characters of the isolates tested were as follows:

Colony reverse color: has varying shades of faint yellow, orange to colourless; conidial heads: short columnar or rarely radiate; conidiophores: brown pigmented, commonly sinuous with smooth wall, ranging from 20 to 210µm,

Table 1. — Production of sterigmatocystin by species of *A. nidulans* group cultivated on Czapek's medium.

Species	No of isolates tested	No of isolates producing sterigmatocystin	Sterigmatocystin produced (mg/l)	
			Minimum	Maximum
A. nidulans (Eidam) Wint.	15	15	48	495
A. nidulans var. *latus* Thom & Raper	10	10	114	136
A. nidulans var. *dentatus* Sandhu & Sandhu	5	0	—	—
A. nidulans var. *acristatus* Fennell & Raper	10	0	—	—
A. quadrilineatus Thom & Raper	15	15	12	357
A. rugulosus Thom & Raper	5	5	243	330
A. violaceus Fennell & Raper	5	5	18	48

usually 70 to 110μm in length; vesicles: usually globose or subglobose, less commonly flask-shaped and ranging from 4 to 16.5μm in diameter; conidia: globose, 3-4.5μm in diameter; hülle cells: globose, subglobose or subelongate ranging from 80 to 220μm in diameter, commonly 120 to 140μm; ascospores: purple-red, lenticular, smooth walled with two equatorial crests, 3.7 to 4.9 x 3.7 to 4.1μm.

Three isolates of *A. nidulans* (No 1107, 1110 and 1115*), produced the largest amounts of sterigmatocystin with the highest stability of production among all the isolates tested (472-295 mg/l). These isolates were characterized by a colorless reverse of colony; conidiophores: short ranging from 20 to 65μm in length; conidia usually globose, and 3-3.5μm in diameter; hülle cells: numerous, 6.6-12.7μm in diameter.

25 isolates belonging to three varieties of *A. nidulans* were tested namely, *A. nidulans* var. *latus* Thom et Raper, *A. nidulans* var. *dentatus* Sandhu et Sandhu and *A. nidulans* var. *acristatus* Fennell et Raper. All 10 isolates belonging to *A. nidulans* var. *latus* Thom et Raper produced sterigmatocystin when grown on Czapek's medium, the production ranging from 114 to 136 mg/l.

On the contrary, none of the 5 isolates of *A. nidulans* var. *dentatus* and 10 of *A. nidulans* var. *acristatus* tested produced any detectable amount when grown on any culture medium, at any incubation temperature (20, 28 and 35°C) or after any incubation period (7, 15 and 21 days) used in this study.

A. quadrilineatus Thom et Raper was represented by 15 different isolates. All isolates produced sterigmatocystin with amounts ranging from 12 to 357 mg/ l. The morphological characters of the isolates tested are as follows: reverse of colony : varying in color from yellow to orange or purple; conidial heads : short, columnar or radiate, green in color; conidiophores: sinuate, smooth walled,

* No of the isolates in the Culture Collection of this laboratory.

pale to dull brownish in color, and 10 to 155μm in length; vesicles: globose to subglobose ranging from 3.3 to 16.5μm in diameter; conidia: globose, and 2.7 to 4μm in diameter; cleistothecia: spherical ranging from 100 to 180μm in diameter; hülle cells: globose to subglobose, 9.9-23μm in diameter and present in all strains varying from few to numerous; ascospores : purple-red, lenticular, smooth wall with four equatorial crests and with spore body ranging from 4 to 4.6 by 3.4 to 3.7μm.

The highest sterigmatocystin production (334-357 mg/l) was obtained with two isolates (No. 1531 and 1534*) which are characterized by faint yellow reverse colony; conidial heads: short and ranging from 40 to 50 by 18 to 30μm; conidiophores: short and ranging from 10 to 55μm in length; vesicles: globose and very small, ranging from 3.3 to 6.6μm in diameter; conidia: globose ranging from 2.7 to 3.1μm in diameter; hülle cells numerous, 9.9-15μm in diameter.

A. rugulosus Thom et Raper and *A. violaceus* were represented each by 5 isolates. All isolates of both species produced sterigmatocystin when cultivated on Czapek's medium. The isolates of *A. rugulosus* produced larger quantities of sterigmatocystin (243-330 mg/l) than those of *A. violaceus* (18-48 mg/l).

IV. – DISCUSSION

The present results revealed that all isolates of *A. nidulans* and *A. rugulosus* produced sterigmatocystin. These findings agree with the earlier results of MISLIVEC et al. (1972) and SCHROEDER and KELTON (1975), who reported that all isolates tested of *A. nidulans* and *A. rugulosus* produced sterigmatocystin.

A. nidulans var. *latus*, and *A. violaceus* have no previous history of toxicogenicity. Production of sterigmatocystin by these members of *A. nidulans* group is reported for the first time.

Sterigmatocystin has never been detected when different isolates of *A. nidulans* var. *dentatus* and *A. nidulans* var. *acristatus* were cultivated on three culture media at different temperatures and the activity assayed after different incubation periods. Therefore they can be considered unable to produce the specific enzyme(s) for sterigmatocystin synthesis.

Sterigmatocystin was reported as a precursor of aflatoxin. HSIEH et al. (1973) showed that resting cells of *A. parasiticus* Speare (*A. flavus* group) efficiently converted sterigmatocystin to aflatoxin B_1. However aflatoxin production was not observed in any of the sterigmatocystin-producing members of the *A. nidulans* group tested in this or earlier studies. This seems to be a common character of sterigmatocystin-producing members of *A. nidulans* group. SCHROEDER & KELTON (1975) reported that *A. nidulans* and *A. rugulosus* (sterigmatocystin-producers) are lacking the enzymes required for the bioconversion of sterigmatocystin to aflatoxin.

Sterigmatocystin may be considered of more concern that previously believed because of the ability of several species to produce this toxin in substantial amounts.

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